# CHAPTER - 1

# INTRODUCTION

Our green planet has been contaminated from day to day by different contaminants. One of the most serious contaminant groups is the heavy metals. There has been increased concern surrounding exposure to heavy metals due to the evolving understanding of their role in the development of cancer. These elements have the ability to induce a number of adverse health effects. Our ecosystem has been contaminated by high concentration of heavy metals released into the biosphere by human activity. Industrial activities, energy production, construction, urban waste treatment, and vehicle exhaust are some of the sources causing large quantities of heavy metal contamination in atmosphere, water and soil.

The heavy metals that are responsible for contamination are

1) Aluminium

2) Arsenic

3) Beryllium

4) Cadmium

5) Nickel

6) Lead

7) Mercury

8) Radium

Out of this heavy metals this project deals with lead collected from river water samples.

Lead is a toxic heavy metal and its exposure constitutes significant risks to health and has been listed as one of the human carcinogens by the International Agency for Research on Cancer (IARC) since 1980.One common source of environmental pollution has been found in the water and soil, which can enter the human food cycle through contaminant produced. It is being banned from its commercial use due to its high contribution of emission of lead to the atmosphere. Certain occupations also play a role in lead exposure, such as mining. Its effects will be more severe by synergistic behaviour of arsenic and lead. One of the molecular mechanisms for toxicity of lead is oxidative stress. By the effect of lead, reactive oxygen species (ROS) levels increase via elevated intracellular $Ca^{2+}$. There are different types of lead sources causing food, water contamination and air pollution by industrial emission.

Forensic science pertains to science applied to the law including criminal investigation with results being presented as evidence in the law courts. In crime investigation, an evidence appears before the investigation agencies, courts and the jury in various forms on daily basis. The ability to recognize, collect and use evidence in criminal investigations determines the success and failure of the case investigators. It is a unique scientific endeavour different from many conventional fields of science, which have been embraced by court of law. It is devoted to the use of scientific methods and procedures together with objective information which will be used to help in solving a crime.

This project comes under the field forensic toxicology, a sub branch of forensic chemistry which applies the field of toxicology and its disciplines such as Analytical Chemistry, Pharmacology and Clinical Chemistry to aid medical or legal investigation of death, poisoning and drug use. The significance of this project in the field of forensics is to find out the quantity of lead exposure in river water samples which is being used by number of human beings that can cause devastating effects on human body. Lead can be found in natural resources like water by the release of the industrial waste like electronic, chemical waste by the industries located in areas near river. This water is being consumed by people in the village areas and due to this number of diseases are widely spread causing cancer, organ functional diseases etc which later lead to death.

Lead toxicity is an important environmental disease and its effects are devastating. There is almost no function in the human body which is not affected by lead toxicity. Lead is being used because of its unique physical and chemical properties that make it suitable for a large number of applications for which humans have exploited its benefits from historical times and thus it has become a common environmental pollutant. Lead is highly persistent in the environment and because of its continuous use its levels rise in almost every country, posing serious threats. Toxic effects of lead affects the renal, reproductive and nervous system. Finally the techniques available for treating lead toxicity are presented with some recent update.

Due to the adverse health effects of lead, detection of trace amount of the element in any environmental sample related with human being is very important. Determination of the element requires sufficiently sensitive techniques for detection at the µg/L or sublevels. In the literature, there are many techniques including atomic absorption spectrometry (AAS),Graphite Furnace Atomic Absorption Spectrometry (GFAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) for the determination of Pb in different matrices. Although AAS has been used for many elements in variety of matrices there is sensitivity problem for many elements in the case of using this instrument. Due to the high sensitivity, low cost, and easy operation, GFAAS has beenpopularly used in literature for Pb. The main purpose of this study was to analyse river water samples for their Pb content by using optimized GFAAS system.

**Figure 1.1 Periyar River**

# CHAPTER - 2

# LITERATURE REVIEW

## 2.1 Interactions of lead(II) with natural river water:

C.M.S Botelho(1994):A comparative study of dissolved complexants for lead(II) on three different sites along a polluted river was undertaken. Intrinsic and apparent acidity constants were obtained from potentiometric titrations. The difference between the two values in all samples reflects conformational and electrostatic effects during titrations. Complexometric capacities for Pb(II) were estimated and average equilibrium constants were obtained from DPASV voltammetric titration curves. Differential equilibrium functions were defined. At the pH measured in the river, the affinity of metal ions for ligands decreases with increasing metal loading of solutions, due to the heterogeneous effect of the ligands.

## 2.2 Lead in Nile River water and finished drinking water in Greater Cairo, Egypt:

M.A.M Mohamed(1998):Lead were measured in raw Nile River water and in finished drinking water at four treatment plants in Greater Cairo, Egypt. Samples were collected monthly during the period September 1993 to August 1994. The river water mean concentration is 29.6 ± 8.74 µg/L for lead. These levels were 14 times less than levels reported in a study published in 1995 (Gomaa 1995). In the drinking water, the mean is9.93 ± 0.5 µg/L for lead. The drinking water mean concentrations were below internationally-recognized drinking water standards. Comparison of the raw and finished drinking water data showed that the water treatment facilities reduced concentrations of lead by 33%.

## 2.3 Determination of lead content of water samples from tabakhane, akkaya dam and nigde-turkey after pre-concentration on activated carbon:

Narin(1999): The concentration of lead, nickel, copper and cadmium in the water samples collected from Tabakhane River and Akkaya Dam (Nigde City-Turkey) have been determined by graphite furnace atomic absorption spectrometry after preconcentration and separation by sorbing the metal/4-(2-Thiazolylazo) resorcinol complexes on an activated carbon column. The concentration ranges of lead, copper and cadmium were 1.1-4.9 mu g/l, 0.7-2.5 mu g/l and 0.3-1.3

mu g/l respectively. The nickel content of the samples was below 1 mu g/l. The metal contents of the water samples were below the values given in the Water Pollution Control Regulation of Turkish Authorities. The metal contents of the sediment samples have been also determined by flame atomic absorption spectrometry after digestion procedure with aqua regia.

### 2.4 Determination of lead in water samples by flame atomic absorption spectrometry after cloud point extraction:

Jian rongchenet.al(2001) determined lead in water samples by Cloud point extraction (CPE) which has been used for the simultaneous pre-concentration of lead after the formation of a complex with 1-(2-thiazolylazo)-2-naphthol (TAN) and later analysis by Flame Atomic Absorption Spectrometry (FAAS) using octylphenoxypolyethoxyethanol(Triton X-114) as surfactant. The chemical variables affecting the separation phase and the viscosity affecting the detection process were optimized. At pH 8.6, pre-concentration of only 50 ml of sample in the presence of 0.05% Triton X-114 and $2\times10^{-5}$ mol $l^{-1}$ TAN permitted the detection of 1.1 lead. The enhancement factor for lead is 55.6 .The proposed method has been applied to the determination of lead.

### 2.5 Determination of Pb in river water samples by inductively coupled plasma optical emission spectrometry after ultrasound-assisted co-precipitation with manganese dioxide

Marcia Sousa Bispo(2005):A simple and efficient procedure for separation and pre-concentration using ultrasound-assisted co-precipitation with manganese dioxide was developed for Pb determination by inductively coupled plasma optical emission spectrometry (ICP OES). The optimization process was carried out using a two-level factorial design and a Doehlertmatrix. Three variables (i.e. concentration of oxidizing solution—$KMnO_4$, concentration of $MnSO_4$ solution and time of ultrasonic irradiation) were used as factors in the optimization. The recoveries, based on the analysis of spiked samples, were between 90% and 105%, and the precision was ≤ 5%. The detection limit and quantification limit for Pb determination were 3.2 and 10.7 $\mu g\ L^{-1}$, respectively. The proposed method was applied for the determination of Pb in water samples from a river heavily polluted by industrial effluents. The recovery measured by analyte addition technique showed that the proposed pre-concentration method had good accuracy.

## 2.6 Determination of Lead in River Water by Graphite-Furnace Atomic Absorption Spectrometry after Coprecipitation with Terbium Hydroxide

Tomoharu MINAMI et.al(2005) : Co-precipitation with terbium hydroxide quantitatively recovered trace amounts of lead(II) at pH 8.7 - 11.5. The precipitate was dissolved in 0.85 mol $dm^{-3}$ nitric acid, and the analytes were determined by graphite-furnace atomic absorption spectrometry (GF-AAS). The presence of terbium (up to 7 g $dm^{-3}$) did not interfere with the determination. The detection limit for lead is 0.5 µg $dm^{-3}$ when the analytes in 200 $cm^3$ of the sample solution 10 $cm^3$. The ions added to river water were quantitatively recovered. Lead in contaminated were concentrated into river water were successfully determined.

## 2.7 A novel slurry sampling analysis of lead in different water samples by electrothermal atomicabsorption spectrometry after co-precipitated with cobalt/pyrrolidinedithiocarbamate complex:

A.Baysalet.al(2008):A preconcentration/separation technique based on the coprecipitation of lead with cobalt/pyrrolidinedithiocarbamate complex ($Co(PDC)_2$) and subsequently its direct slurry sampling determination by electrothermal atomic absorption spectrometry (AAS) was described. For this purpose, at first, lead was co-precipitated with cobalt/pyrrolidinedithiocarbamate complex formed using ammonium pyrrolidinedithiocarbamate (APDC) as a chelating agent and cobalt as a carrier element. The supernatant was then separated and the slurry of the precipitate prepared in Triton X-100 was directly analyzed by electrothermal atomic absorption spectrometry with respect to lead concentration. The effects of experimental conditions on coprecipitation of lead with gathering precipitate as well as homogeneity and stability of the slurry were investigated. After the optimization of experimental parameters, a 100-fold enrichment of the analyte with quantitative recovery (>90%) and high precision (<10% R.S.D.) were obtained. By using the proposed technique, the lead concentrations in heavy matrices of Certified Sea-water and wastewater samples could be practically and rapidly determined in the range of 95% confidence level. The detection limit of the described method for lead using sample-matching blanks was 1.5 ng/L ($3\sigma$, N = 10*)*

## 2.8 On-line sequential injection dispersive liquid–liquid microextraction system for flame atomic absorption spectrometric determination of lead in water samples:

Aristidis N. Anthemidiset.al(2009) used a simple and powerful on-line Sequential Injection (SI) Dispersive Liquid–Liquid Micro-extraction (DLLME) system which was developed as an alternative approach for on-line metal pre-concentration and separation, using extraction solvent at micro litre volume. The potentials of this novel schema, coupled to Flame Atomic Absorption Spectrometry (FAAS), were demonstrated for trace of lead determination in water samples. The stream of methanol (disperser solvent) containing 2.0% (v/v) xylene (extraction solvent) and 0.3% (m/v) ammonium diethyldithiophosphate (chelating agent) was merged on-line with the stream of sample (aqueous phase), resulting a cloudy mixture, which was consisted of fine droplets of the extraction solvent dispersed entirely into the aqueous phase. By this continuous process, metal chelating complexes were formed and extracted into the fine droplets of the extraction solvent. The hydrophobic droplets of organic phase were retained into a microcolumn packed with PTFE-turnings. A portion of 300_L isobutylmethylketone was used for quantitative elution of the analytes, which transported directly to the nebulizer of FAAS. All the critical parameters of the system such as type of extraction solvent, flow-rate of disperser and sample, extraction time as well as the chemical parameters were studied. Under the optimum conditions the enhancement factor for lead was 265.For lead, detection limit and precision were 0.54_gL−1 and 1.9% at 30.0_gL−1 Pb(II). The developed method was evaluated by analyzing certified reference material and applied successfully to the analysis of environmental water samples.

## 2.9 A new amine based microextraction of lead (II) in real water samples using flame atomic absorption spectrometry:

M.A Habila(2019): A new amine based microextraction procedure has been established for the separation and preconcentration of lead (II). N,N-dimethyl-n-octylamine was used as extraction solvent and chelating agent for lead (II). Micro-sampling FAAS was used for the determination of lead. The analytical parameters including pH, amounts of N,N-dimethyl-n-octylamine, sample volume etc. were optimized. The influences of the matrix components were also investigated. The validation of the procedure was done by the analysis of TMDA 64.2 certified reference material. The Limit of detection (LOD) of developed method was 2.6 μg $L^{-1}$. The developed microextraction procedure was successfully applied to natural water samples.

### 2.10 Sequential drinking water sampling as a tool for evaluating lead in flint, Michigan:

Darren A Lytle(2019):Eliminating the sources of human lead exposure is an ongoing public health goal. Identifying the make-up of household plumbing and service line material type is important for many reasons including understanding lead release sources and mechanisms, targeting locations for lead service line (LSL) removal, and assessing the effectiveness of lead remediation strategies. As part of the response to Flint, Michigan's drinking water lead public health crisis, a return to their original drinking watersource (Lake Huron) and an increase in orthophosphate dose was implemented in late 2015. In 2016, EPA performed multiple rounds of sequential or “profiling” water sampling to evaluate corrosion control effectiveness  and identify lead sources in homes and service lines, as well as to evaluate the effectiveness of corrosion control treatment with time on the different plumbing components. The results showed that lead levels, including high lead levels likely associated with particles, decreased with time in homes sampled during the 11-month evaluation period.

# CHAPTER – 3

# AIM AND OBJECTIVES

## 3.1 AIM:

Analysis of lead in river water samples collected from different spots of Periyar River in Kerala.

## 3.2 OBJECTIVES:

- To analyse whether lead is present in Periyar river water which is being used by number of people in nearby area.
- If present, the quantity of lead content is to be analysed.
- If the measured quantity is less, Is it suitable for human use?
- If the measured quantity exceeds the minimum, whether or not it leads to several organ functional diseases or cancer?

# CHAPTER - 4

# MATERIALS AND METHODOLOGY

## 4.1 Materials Required:

1) Collected sample

2) Plastic vials

3) Filter paper

4) Funnel

5) PerkinElmer Atomic Absorption Spectrometer PinAAcle 900H

## 4.2: Instrument

**Figure 4.2 PerkinElmer Atomic Absorption Spectrometer PinAAcle 900H**

The technique used in the present study is Graphite furnace Atomic Absorption Spectrometer and the basic principle is “Every element has a specific number of electrons associated with itsnucleus. The normal and most stable orbital configuration of an atom is the ground state. If energy is applied to an atom, the energy will be absorbed of a specific wavelength and an outer electron will be promoted to a less stable configuration known as the excited state. Since this state is unstable,the atom will immediately return to the ground state, releasing light energy. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources andcareful selection of wavelengths allow the specific determination of individual elements”.

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest.
2. An "Absorption Cell" in which atoms of the sample are produced( Graphite furnace).
3. A Monochromator for light dispersion.
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading after it has been processed by the instrument electronics.

### 4.3 Methodology:

The four water samples were collected from different parts of Periyar River.Then the samples were filtered with funnel into 50ml plastic vials and then it is transferred to 2ml vials for analysis using the instrument Atomic Absorption Spectrometer and the technique used is Graphite Furnace. Graphite Furnace Atomic Absorption Spectrometer is capable of detecting minute traces i.e, ppt (parts per thousand) of metals especially heavy metals. As the sample is a liquid, it can be directly analysed in graphite furnace.

# CHAPTER-5

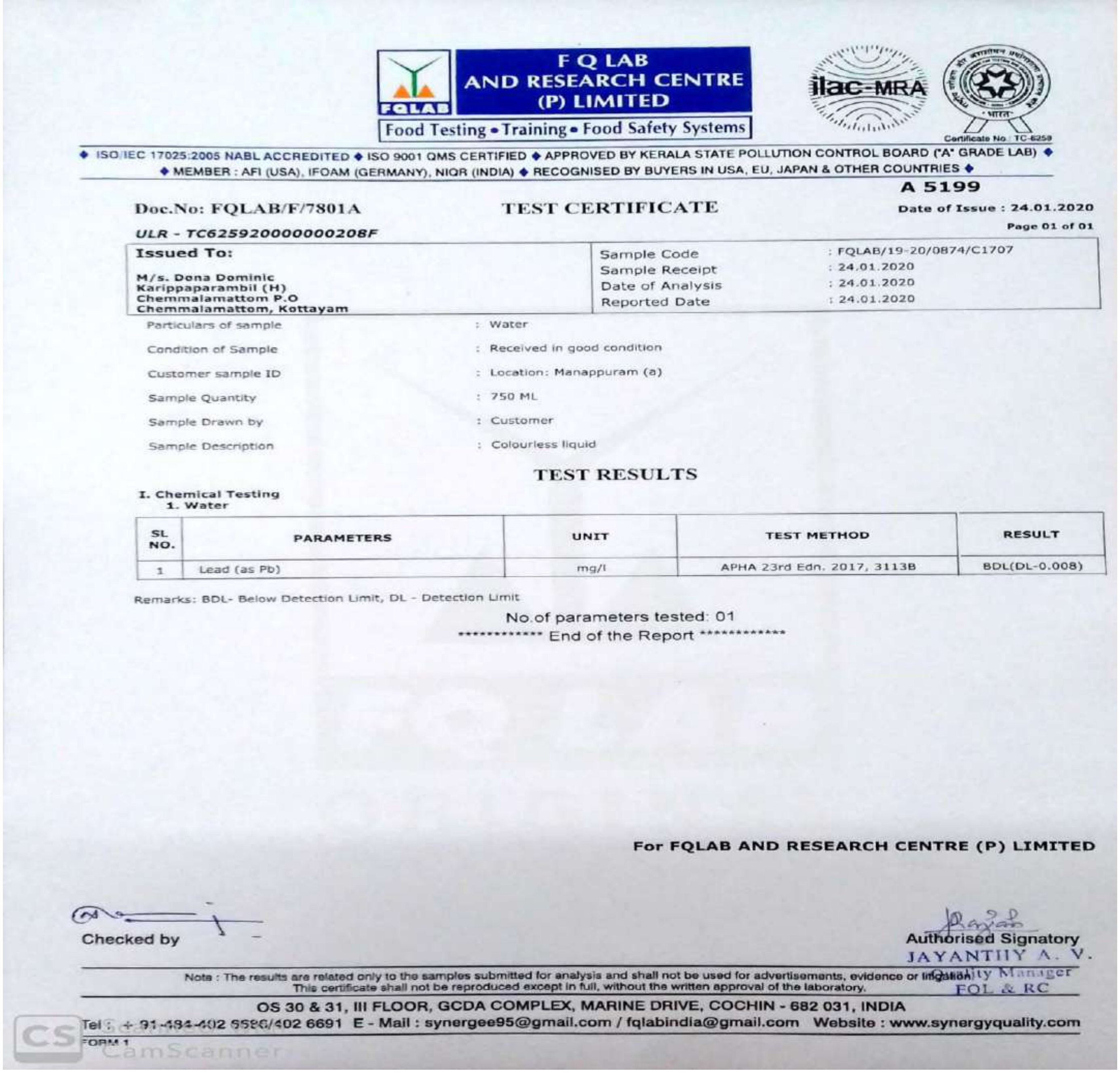
## OBSERVATION

Sample 1 :

SampleID:FQLAB/19-20/0874/C1707

| Analyte | Corr. Absorbance | Conc. (Calib) | Std.Dev | Conc. (sample) | Std.Dev | %RSD |
|---|---|---|---|---|---|---|
| Pb283.31 | | | | | | |
| | -0.0176 | -3.577 | µg/L | -3.577 | µg/L | |
| | -0.0179 | -3.650 | µg/L | -3.650 | µg/L | |
| | | | | | | |
| Mean | -0.0177 | -3.613 | 0.0520µg/L | -3.613 | 0.0520µg/L | 1.4397 |

**Table 5.1-Sample 1**



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**Chemmalamattom P.O**
**Chemmalamattom, Kottayam**

Sample Code : FQLAB/19-20/0874/C1707
Sample Receipt : 24.01.2020
Date of Analysis : 24.01.2020
Reported Date : 24.01.2020

Particulars of sample : Water
Condition of Sample : Received in good condition
Customer sample ID : Location: Manappuram (a)
Sample Quantity : 750 ML
Sample Drawn by : Customer
Sample Description : Colourless liquid

**TEST RESULTS**

**I. Chemical Testing**
**1. Water**

| SL NO. | PARAMETERS | UNIT | TEST METHOD | RESULT |
|---|---|---|---|---|
| 1 | Lead (as Pb) | mg/l | APHA 23rd Edn. 2017, 3113B | BDL(DL-0.008) |

Remarks: BDL- Below Detection Limit, DL - Detection Limit

No.of parameters tested: 01

************ End of the Report ************

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FORM 1

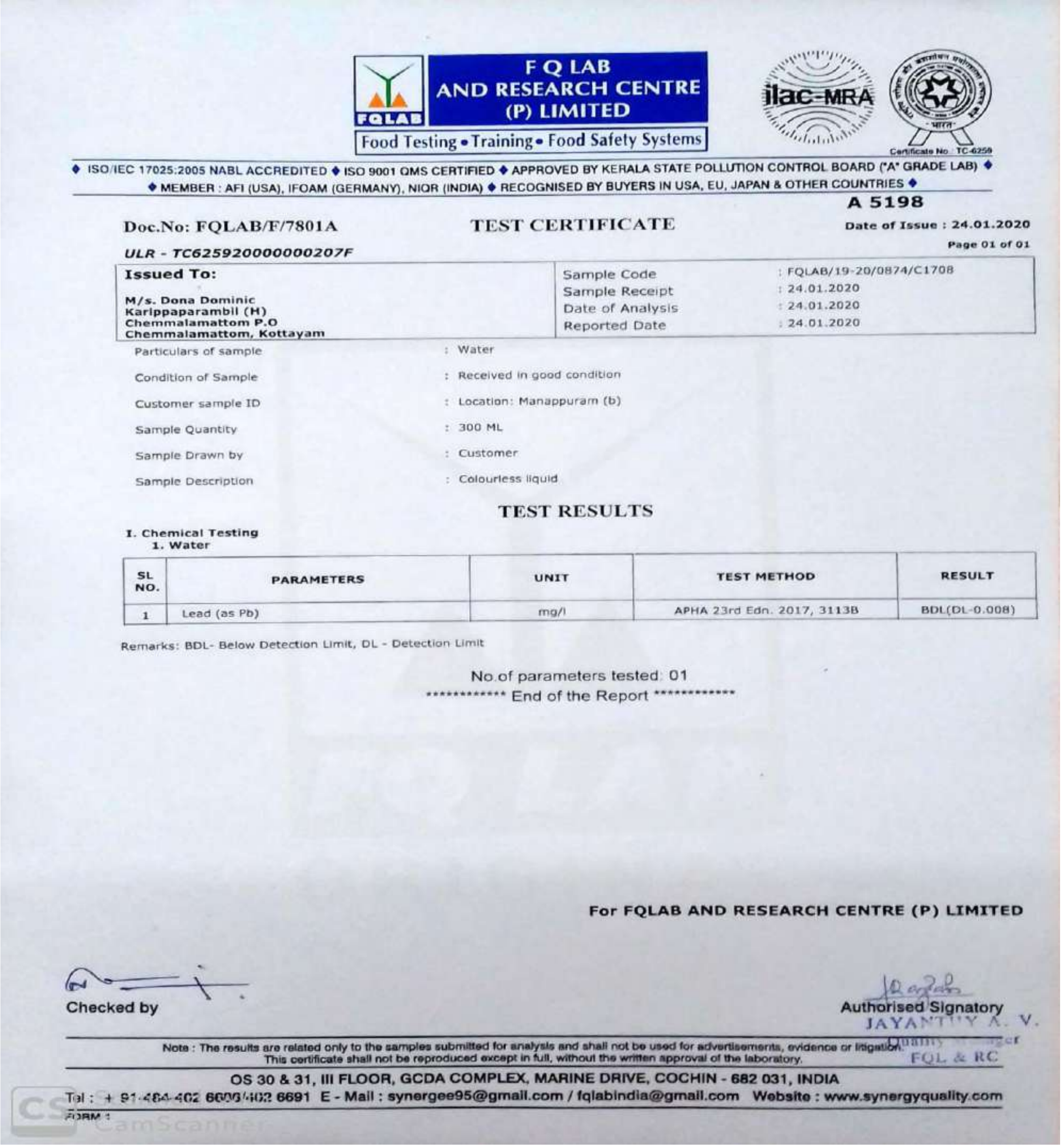
**5.1-Report of sample 1**

Sample 2:

Sample ID:FQLAB/19-20/0874/C1708

| Analyte | Corr.(Absorbance) | Conc.(Calib) | Std.Dev | Conc(sample) | Std.Dev | %RSD |
|---|---|---|---|---|---|---|
| Pb283.31 | | | | | | |
| | -0.0144 | -2.930 | µg/L | -2.930 | µg/L | |
| | -0.0138 | -2.805 | µg/L | -2.805 | µg/L | |
| | | | | | | |
| Mean | -0.0141 | -2.867 | 0.0888µg/L | -2.867 | 0.0888µg/L | 3.0980 |

**Table 5.2 – Sample 2**



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| | |
|---|---|
| Particulars of sample | : Water |
| Condition of Sample | : Received in good condition |
| Customer sample ID | : Location: Manappuram (b) |
| Sample Quantity | : 300 ML |
| Sample Drawn by | : Customer |
| Sample Description | : Colourless liquid |

**TEST RESULTS**

**I. Chemical Testing**
**1. Water**

| SL NO. | PARAMETERS | UNIT | TEST METHOD | RESULT |
|---|---|---|---|---|
| 1 | Lead (as Pb) | mg/l | APHA 23rd Edn. 2017, 3113B | BDL(DL-0.008) |

Remarks: BDL- Below Detection Limit, DL - Detection Limit

No.of parameters tested: 01
************* End of the Report *************

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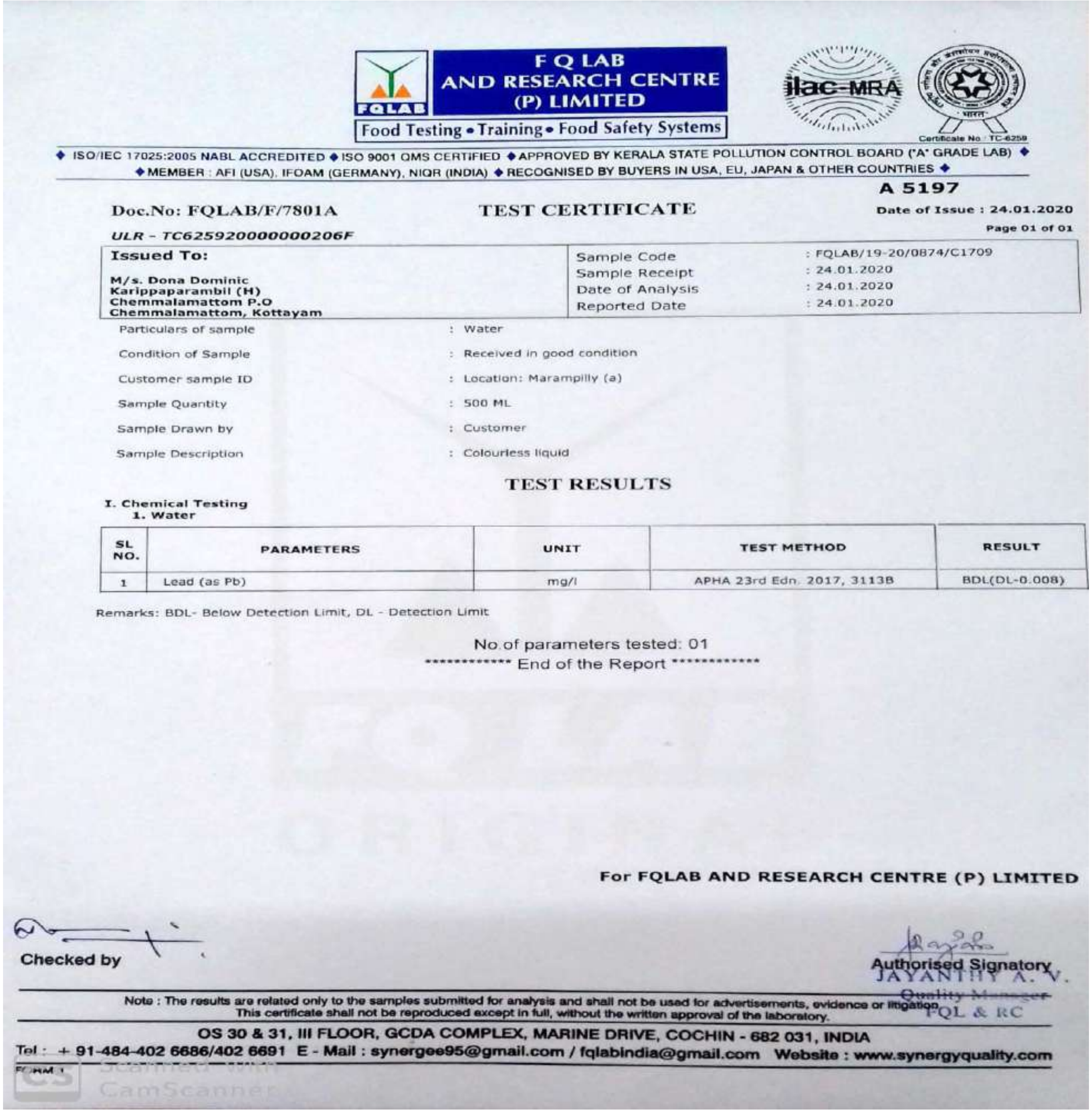
5.2-Report of sample 2

Sample 3 :

Sample ID : FQLAB/19-20/0874/C1709

| Analyte | Corr.(Absorbance) | Conc.(Calib) | Std.Dev | Conc(sample) | Std.Dev | %RSD |
|---|---|---|---|---|---|---|
| Pb 283.31 | | | | | | |
| | -0.0156 | -3.170 | µg/L | -3.170 | µg/L | |
| | -0.0131 | -2.667 | µg/L | -2.667 | µg/L | |
| | | | | | | |
| Mean | -0.0143 | -2.919 | 0.3556µg/L | -2.919 | 0.3556µg/L | 12.1836 |

**Table 5.3-Sample 3**



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Particulars of sample : Water
Condition of Sample : Received in good condition
Customer sample ID : Location: Marampilly (a)
Sample Quantity : 500 ML
Sample Drawn by : Customer
Sample Description : Colourless liquid

**TEST RESULTS**

**I. Chemical Testing**
**1. Water**

| SL NO. | PARAMETERS | UNIT | TEST METHOD | RESULT |
|---|---|---|---|---|
| 1 | Lead (as Pb) | mg/l | APHA 23rd Edn. 2017, 3113B | BDL(DL-0.008) |

Remarks: BDL- Below Detection Limit, DL - Detection Limit

No.of parameters tested: 01
************* End of the Report *************

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## 5.3-Report of sample 3

## Sample 4:

Sample ID : FQLAB/19-20/0874/C710

| Analyte | Corr.(Absorbance) | Conc.(Calib) | Std.Dev | Conc.(sample) | Std.Dev | %RSD |
|---|---|---|---|---|---|---|
| Pb283.31 | | | | | | |
| | -0.0141 | -2.864 | µg/L | -2.864 | µg/L | |
| | -0.0130 | -2.646 | µg/L | -2.646 | µg/L | |
| | | | | | | |
| Mean | -0.0135 | -2.755 | 0.1544 µ/L | -2.755 | 0.1544µg/L | 5.6053 |

**Table 5.4-Sample 4**

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Particulars of sample : Water
Condition of Sample : Received in good condition
Customer sample ID : Location: Marampilly (b)
Sample Quantity : 500 ML
Sample Drawn by : Customer
Sample Description : Colourless liquid

**TEST RESULTS**

**I. Chemical Testing**
**1. Water**

| SL NO. | PARAMETERS | UNIT | TEST METHOD | RESULT |
|---|---|---|---|---|
| 1 | Lead (as Pb) | mg/l | APHA 23rd Edn. 2017, 3113B | BDL(DL-0.008) |

Remarks: BDL- Below Detection Limit, DL - Detection Limit

No.of parameters tested: 01
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**5.4-Report of sample**

# CHAPTER –6

# RESULT AND CONCLUSION

## 6.1 Result:

The four samples collected from four different locations of Periyar River is analyzed for the presence of Lead. The **actual detection limit is to be 0.008** and the  Mean result analyzed for the samples are

Sample ID: FQLAB/19-20/0874/C1707: -3.613

Sample ID: FQLAB/19-20/0874/C1708: -2.867

Sample ID: FQLAB/19-20/0874/C1709: -2.919

Sample ID: FQLAB/19-20/0874/C1710: -2.755, which shows that the values are below the detection limit.

As the values are below the detection limit, the samples analyzed do not contain the heavy metal lead.

## 6.2 Conclusion:

Of all the Heavy Metals, Lead appears to be rather prominent. The use of lead has been evidenced from ancient times and its toxicity reports are well documented. Due to its important Physico-chemical properties, it has been used all over the world. With the onset on industrialisation from the seventeenth century onwards, its use increased manifold, leading to increased toxicity in humans. Lead toxicity is evident from the literature and there is almost no function in the body which is not affected by lead. Lead toxicity disrupts the functions of the digestive system, nervous system, respiratory system, reproductive system etc.

The toxicologist have continually detected the heavy metal concentration in various water bodies, plants, vegetables etc. Human health is directly affected by the consumption of polluted water, fish, fruits, vegetables and plants which are the main source of food for humans. The levels of heavy metal contamination in water is increased. Variety of heavy metals, some of them are potentially toxic and are transferred to the surrounding environment through different pathways. Water may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal, Electronic waste.

The sample collected from different spots of periyar river does not contain the presence of lead.So it is suitable for human use.Periyar river is the longest river and the river with the largest discharge potential in the Indian state of Kerala.It provides drinking water for several major towns.ThePeriyar is of utmost significance to the economy of Kerala.It generates a significant proportion of Kerala's electrical power via the Idukki Dam and flows along a region of industrial and commercial activity. The river also provides water for irrigation and domestic use throughout its course besides supporting a rich fishery. This study has proved that the heavy metal lead is not present in the river and is suitable to use for different purposes.

Further study can be done on other types of Heavy Metals present in Periyar River by using AAS.

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